

## The Influence of Fructose and its Metabolites on Ethanol Metabolism *in vitro*

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(Received 24 May 1966)

1. Fructose caused an increase in the rate of ethanol oxidation by rat-liver slices, and D-glyceraldehyde was found to have a similar effect. 2. Addition of glycerol lowered the rate of ethanol oxidation if the incubation medium contained fructose and ethanol, but no such effect was found if it contained glucose and ethanol. 3. The formation of glycerol by the slices during incubation and the concentration of  $\alpha$ -glycerophosphate in the slices were highest in medium containing fructose and ethanol. 4. In experiments without ethanol in the incubation medium, fructose strongly increased the pyruvate concentration, which resulted in a decrease of the lactate/pyruvate concentration ratio. Addition of ethanol to the medium resulted in a marked decrease in pyruvate concentration. 5. Oxygen consumption is greater in slices incubated in medium containing fructose and ethanol than in slices incubated in medium containing glucose and ethanol.

The rate of ethanol oxidation in the liver is generally accepted as being relatively constant. The only substance so far known to produce a considerable increase in the rate of ethanol oxidation *in vivo* is fructose. This was shown by Stuhlfauth & Neumaier (1951) and confirmed by Pletscher, Bernstein & Staub (1952) and by Lundquist & Wolters (1958).

Pyruvate has been reported to cause a large increase in the ethanol oxidation in infusion experiments with dogs (Westerfeld, Stotz & Berg, 1943), but other investigators have failed to confirm this effect (Smith & Newman, 1959). In rat-liver slices pyruvate, however, effectively increases the rate of ethanol oxidation (Leloir & Muñoz, 1938; Smith & Newman, 1959).

Ethanol oxidation is started by the oxidation of ethanol to acetaldehyde, which is further oxidized to free acetate (Lundquist, Tygstrup, Winkler, Mellemsgaard & Munck-Petersen, 1961). Both processes require NAD. The oxidation of ethanol to acetaldehyde involves a number of partial reactions. Of these the dissociation of the alcohol dehydrogenase-NADH complex is the rate-limiting factor, as shown by Theorell & Chance (1951). Holzer & Schneider (1955) have proposed that the accelerating effect of fructose on the rate of ethanol oxidation is due to a direct oxidation of this alcohol dehydrogenase-NADH complex by means of a fructose metabolite, namely glyceraldehyde.

In the present paper the effects of fructose and D-glyceraldehyde on ethanol metabolism in rat-

liver slices are reported. A preliminary account of this work has been published (Thieden, 1965).

### MATERIALS AND METHODS

Wistar rats weighing 100–200 g. were used. They were fed *ad libitum* but deprived of food for 20 hr. before use. The rats were killed by a blow on the head and decapitated. The livers were rapidly removed and chilled in ice. Slices 0.3 mm. thick were cut with a McIlwain-Buddle tissue chopper at 4° and transferred to prepared Erlenmeyer flasks containing 10 ml. of cold incubation medium or to Warburg manometer flasks containing 3 ml. of medium. The amount of tissue is estimated by weighing the flasks before and after addition of slices. Each flask contained 0.3–0.7 g. of tissue.

The incubation medium consisted of a phosphate buffer (Robinson, 1949; DeLuca & Cohen, 1964) at pH 7.4. This medium was used with the additions indicated in the Tables. It was prepared from purest grade reagents and concentrations of the ions in this medium were: Na<sup>+</sup>, 140 mm; K<sup>+</sup>, 5 mm; Ca<sup>2+</sup>, 2.5 mm; Cl<sup>-</sup>, 144 mm; phosphate, 3 mm; SO<sub>4</sub><sup>2-</sup>, 1 mm. The flasks were shaken in a thermostatically controlled water tank at 37° and gassed with oxygen for 5 min. at the beginning of the incubation. Samples of the incubation medium for determination of ethanol were removed at 15 min. intervals and the total incubation period was 90 min. The reaction was stopped by addition of HClO<sub>4</sub> and the content of ethanol determined enzymically (Lundquist, 1959).

The same liver was used in parallel assays and all results are referred to the wet weight of liver. The relative changes are used in determination of the probability limits. In this way the possible difference in the ethanol oxidation capacities of the different livers will not distort the experimental

findings. Control samples without liver slices showed a small disappearance of ethanol caused by the evaporation and a correction for this was applied. The fall in pH that occurs during the incubation does not affect substantially the rate of ethanol oxidation.

The rate of oxygen uptake by the slices was determined with conventional constant-volume respirometers.

The following determinations were done by the methods indicated: glycerol (Wieland, 1962), pyruvate (Bücher, Czok, Lamprecht & Latzko, 1962) and lactate (Hohorst, 1962a) in the incubation medium, and  $\alpha$ -glycerophosphate (Hohorst, 1962b) in slices and in the incubation medium. In experiments in which these substances were measured the incubation time was 1 hr.

## RESULTS

*Influence of fructose on the rate of ethanol oxidation.* When slices from one liver were incubated in buffer containing ethanol (4mM) and fructose (11mM) or buffer containing the same amount of ethanol and glucose (11mM), a 15% higher rate of ethanol oxidation was observed with fructose (Table 1).

*Influence of D-glyceraldehyde on the rate of ethanol oxidation.* Glyceraldehyde at room temperature is in a dimer (polymer) form (Baer & Fischer, 1939) and, in preliminary experiments, did not increase the rate of ethanol oxidation in comparison with glucose. When the incubation medium containing D-glyceraldehyde was heated at 90° for 4 min. immediately before the experiment, a procedure which dissociates the dimer form, an increase in the oxidation rate was observed. This increase was 18% and was statistically significant (Table 1). The exact concentration of monomer D-glyceraldehyde in our incubation medium is not known, because in the cold a relatively slow reassociation of glyceraldehyde occurs (Baer & Fisher, 1939).

*Influence of glycerol on the rate of ethanol oxidation.* Addition of glycerol (20mM) to an incubation

medium that contained fructose (11mM) and ethanol (4mM) resulted in a reversal of the acceleration caused by fructose (19% decrease). The addition of the same concentration of glycerol to the incubation medium containing glucose instead of fructose gave a decrease in ethanol oxidation of 9%. This decrease was, however, not significant.

*Changes in glycerol content of the incubation medium after incubation with liver slices.* Glycerol is formed from fructose during ethanol oxidation (Tygstrup, Winkler & Lundquist, 1965). In experiments with liver slices the glycerol concentration was higher after incubation in a medium that contained fructose and ethanol than in a medium that contained fructose or glucose alone or that contained glucose and ethanol (Table 2).

*$\alpha$ -Glycerophosphate content of liver slices after incubation.* As rat liver contains an active glycerokinase (Bublitz & Kennedy, 1954) the formation of glycerol may result in the formation of  $\alpha$ -glycerophosphate. The concentration of  $\alpha$ -glycerophosphate in the liver slices was determined immediately before incubation and after incubation of the slices in different media for 1 hr. The highest  $\alpha$ -glycerophosphate concentrations were found before incubation probably because the liver had been anoxic for 2–4 min. (Peterson, Gaudin, Boeck & Beatty, 1964). When determined after incubation the  $\alpha$ -glycerophosphate concentrations were highest in slices incubated in a medium containing fructose and ethanol. No  $\alpha$ -glycerophosphate could be detected in the incubation medium.

*Changes in the lactate/pyruvate ratio during incubation with different substrates.* In experiments without ethanol, fructose strongly increased the pyruvate concentration, which resulted in a decrease of the lactate/pyruvate ratio. The addition of ethanol to the medium resulted in a marked

Table 1. *Effect of fructose, glyceraldehyde and glycerol on the rate of ethanol oxidation*

The rate of ethanol oxidation was measured by incubating liver slices in phosphate buffer containing Na<sup>+</sup> (140mM), K<sup>+</sup> (5mM), Ca<sup>2+</sup> (2.5mM), Cl<sup>-</sup> (144mM), phosphate (3mM) and SO<sub>4</sub><sup>2-</sup> (1mM) at 37° with additions as indicated. The results are expressed as means  $\pm$  s.e.m. with the numbers of determinations given in parentheses. Fisher's *P* values represent the probability of difference between the means (corresponding pairs). Values of *P* greater than 0.05 are shown as N.S., i.e. not significantly different.

Additions to medium	Rate ( $\mu$ moles of ethanol/g. wet wt. of tissue/hr.)	Difference in rates ( $\mu$ moles of ethanol/g. wet wt. of tissue/hr.) and significance ( <i>P</i> )
Glucose (11mM)	25.0 $\pm$ 2.5 (5)	4.8 $\pm$ 1.2 ( <i>P</i> < 0.02)
Fructose (11mM)	29.8 $\pm$ 2.4 (5)	
Glucose (11mM)	25.9 $\pm$ 1.2 (5)	4.8 $\pm$ 1.0 ( <i>P</i> < 0.01)
Glyceraldehyde (11mM)	30.7 $\pm$ 0.8 (5)	
Glucose (11mM)	23.7 $\pm$ 1.4 (4)	2.0 $\pm$ 1.2 (N.S.)
Glucose (11mM) + glycerol (20mM)	21.7 $\pm$ 2.3 (4)	
Fructose (11mM)	31.1 $\pm$ 4.8 (5)	5.5 $\pm$ 1.0 ( <i>P</i> < 0.01)
Fructose (11mM) + glycerol (20mM)	25.6 $\pm$ 2.8 (5)	

Table 2. *Effect of ethanol on the formation of glycerol, lactate and pyruvate from liver slices and on the content of  $\alpha$ -glycerophosphate*

The liver slices were incubated in phosphate buffer at 37° for 1 hr. (details are given in the legend to Table 1). Glycerol, pyruvate and lactate in the medium were determined after this time.  $\alpha$ -Glycerophosphate in the slices was determined before and after the incubation. Additions to the medium were as indicated. Statistical treatment was as in Table 1. The means compared are indicated by the numbers in the first column. The  $\alpha$ -glycerophosphate in slices before incubation was  $1.34 \pm 0.14$  (6)  $\mu$ moles/g. wet wt.

Additions	Glycerol ( $\mu$ mole/g. wet wt./hr.)	Pyruvate ( $\mu$ mole/g. wet wt./hr.)	Lactate ( $\mu$ moles/g. wet wt./hr.)	Lactate/ pyruvate ratio	$\alpha$ -Glycero- phosphate ( $\mu$ mole/g. wet wt./hr.)
(1) Glucose (11 mm)	$0.26 \pm 0.19$ (5)	$0.03 \pm 0.01$ (8)	$1.38 \pm 0.42$ (8)	41.8	$0.09 \pm 0.09$ (6)
(2) Glucose (11 mm) + ethanol (4 mm)	$0.48 \pm 0.17$ (5)	$0.05 \pm 0.01$ (8)	$3.83 \pm 0.60$ (8)	85.0	$0.29 \pm 0.06$ (6)
(3) Fructose (11 mm)	$0.36 \pm 0.15$ (5)	$0.84 \pm 0.17$ (8)	$7.06 \pm 0.90$ (8)	8.4	$0.34 \pm 0.13$ (6)
(4) Fructose (11 mm) + ethanol (4 mm)	$0.93 \pm 0.14$ (5)	$0.11 \pm 0.05$ (8)	$6.11 \pm 0.64$ (8)	56.5	$0.87 \pm 0.10$ (6)
<i>P</i> values:					
(4) versus (1)	< 0.01	< 0.02	< 0.001		< 0.001
(4) versus (2)	< 0.05	< 0.05	< 0.01		< 0.001
(4) versus (3)	< 0.01	< 0.01	N.S.		< 0.01

Table 3. *Effect of ethanol on oxygen consumption*

The rate of oxygen uptake by liver slices incubated in phosphate buffer, with additions as indicated, was determined by using conventional constant-volume respirometers. Statistical treatment was as in Table 1. The means compared are indicated by the numbers in the first column.

Additions	O <sub>2</sub> consumption ( $\mu$ moles/g. wet wt./hr.)
(1) Glucose (11 mm)	$47.7 \pm 5.6$ (6)
(2) Glucose (11 mm) + ethanol (4 mm)	$49.6 \pm 5.0$ (6)
(3) Fructose (11 mm)	$53.1 \pm 5.7$ (6)
(4) Fructose (11 mm) + ethanol (4 mm)	$58.9 \pm 4.7$ (6)
<i>P</i> values:	
(4) versus (1)	< 0.02
(4) versus (2)	< 0.05
(4) versus (3)	N.S.

decrease in pyruvate concentration and an increase of the lactate/pyruvate ratio. Whether there was a change in the lactate/pyruvate ratio after addition of ethanol when the medium contained glucose and no fructose could not be established, as the amount of pyruvate present was too small to be determined with sufficient accuracy. The concentrations of lactate and pyruvate in the incubation medium used in the present experiments were considerably lower than the concentrations found by Forsander (1966), who incubated liver slices of non-starved rats in a bicarbonate buffer. We were, however, able to reproduce his experimental findings by using the stated experimental conditions, namely a bicarbonate buffer and non-starved rats.

*Oxygen consumption.* The oxygen consumption

was about 48  $\mu$ moles/g. wet wt./hr. when liver slices were incubated with a medium containing glucose (Table 3). Addition of ethanol to this medium had no effect on the oxygen consumption. The addition of fructose to the incubation medium instead of glucose resulted in an increase of oxygen consumption, but this change was not significant. If ethanol was added to the medium containing fructose the outcome was a further increase in the oxygen consumption. This increase was significant in relation to oxygen consumption observed with incubation medium consisting of buffer and glucose or buffer, glucose and ethanol.

## DISCUSSION

It is assumed that fructose in the liver becomes phosphorylated to fructose 1-phosphate and then cleaved to dihydroxyacetone phosphate and glyceraldehyde. The finding that D-glyceraldehyde, as well as fructose, accelerates the ethanol metabolism suggests that a part of the accelerating effect of fructose on the ethanol metabolism by rat-liver slices may be caused by processes in which glyceraldehyde takes part. The effect of fructose on ethanol metabolism in human liver slices is much greater; the acceleration is 90% (H. I. D. Thieden, F. Lundquist & A. Schmidt, unpublished work), but we suppose that the underlying mechanism is the same.

The increased rate of glycerol formation when the incubation medium contained both fructose and ethanol and the observation that addition of glycerol lowered the rate of ethanol oxidation if the medium contained fructose and ethanol is consistent with the theory described above. Lamprecht

& Heinz (1958) have shown that glyceraldehyde is oxidized by aldehyde dehydrogenase to glycerate. Holldorf, Holldorf, Schneider & Holzer (1959) found the Michaelis constant of this enzyme for glyceraldehyde to be  $2.8 \times 10^{-4} \text{ M}$ . As the Michaelis constant for the reduction of glyceraldehyde to glycerol by alcohol dehydrogenase is  $3 \times 10^{-2} \text{ M}$  (Holzer & Schneider, 1955), the oxidation of glyceraldehyde to glycerate would appear to be the most probable pathway. Our results are, however, consistent with the findings of Tygstrup *et al.* (1965), who found evidence for the assumption that the oxidation of glyceraldehyde to glycerate is blocked if fructose is metabolized together with ethanol. Their explanation is that acetaldehyde, which is formed from ethanol, competes effectively with glyceraldehyde for aldehyde dehydrogenase.

In the present experiments the addition of fructose to the incubation medium produces a high pyruvate concentration in the medium and a higher  $\alpha$ -glycerophosphate concentration in the slices. Addition of ethanol to medium containing fructose causes a diminished pyruvate formation and a greatly increased  $\alpha$ -glycerophosphate concentration. The glyceraldehyde will not only be oxidized to glycerate, but will also be reduced to glycerol, which may explain the lower pyruvate formation and the increased  $\alpha$ -glycerophosphate formation. Pyruvate is formed presumably from fructose via glycerate (Tygstrup *et al.* 1965).

Addition of ethanol to the medium containing glucose resulted in increased NADH formation, which can explain the increased formation both of lactate and  $\alpha$ -glycerophosphate. The increase of  $\alpha$ -glycerophosphate content of the liver after ethanol administration has also been reported by Nikkilä & Ojala (1963) in experiments *in vivo*. The increase in the concentration of  $\alpha$ -glycerophosphate after addition of ethanol has a parallel in the increase of  $\alpha$ -glycerophosphate concentration during anoxia (Hohorst, Kreutz & Reim, 1961; Ciaccio, Keller & Boxer, 1960). It has been suggested that dihydroxyacetone phosphate under anaerobic conditions can compete with pyruvate as hydrogen acceptor in mammalian tissue (Klingenberg & Bücher, 1960). Several workers (Hohorst *et al.* 1961; Ciaccio *et al.* 1960) have demonstrated that in liver, during oxygen deficit, dihydroxyacetone phosphate does accept hydrogen from cytoplasmic NADH and causes an increase in  $\alpha$ -glycerophosphate.

The increased oxygen consumption of liver slices during incubation in medium containing both ethanol and fructose compared with oxygen consumption in the presence of ethanol and glucose corresponds with results from investigations with infusion of these substances (Tygstrup *et al.* 1965).

This work was supported by grants from Statens almindelige Videnskabsfond and from Carl and Ellen Hertz' Legat til dansk læge- og naturvidenskab. The skilled technical assistance of Mrs E. Thieden is gratefully acknowledged.

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